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Amino acid and vascular endothelial growth factor levels in subretinal fluid in rhegmatogenous retinal detachment

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Purpose: To study the concentrations of amino acids and vascular endothelial growth factor (VEGF) in subretinal fluid (SRF) of cases with rhegmatogenous retinal detachment (RRD). The relevance of the results with postoperative anatomic and functional success in RRD was investigated.

Methods: Fifty-three patients were included in this prospective study. The study group consisted of 46 patients who had scleral buckling surgery with the diagnosis of RRD, and SRF was obtained during the surgery. The control specimens consisted of vitreous samples of seven patients who were diagnosed with pars plana vitrectomy without RRD. Study cases were divided into three groups, corresponding to the duration of retinal detachment. Clinical characteristics, including best corrected visual acuity (BCVA) and anatomic status at month 6, were recorded. Concentrations of 15 selected amino acids were quantified by using high performance liquid chromatography, and VEGF levels were measured with enzyme immunoassay.

Results: When compared with the control group, SRF concentrations of aspartate, citrulline, glutamate, and glycine increased significantly in the study group ($p<0.05$). Statistical analysis showed that concentrations of alanine, isoleucine, leucine, methionine, phenylalanine, threonine, tyrosine, and valine decreased ($p<0.05$). SRF levels of glutamine, taurine, and serine had no significant change. SRF VEGF levels were significantly higher than the vitreous samples of the controls ($p<0.001$). Time-dependent changes and interactions between VEGF and amino acids were observed. There was no correlation between the concentrations of amino acids or VEGF with the parameters of BCVA and anatomical success.

Conclusions: Significant changes occur in concentrations of amino acids and VEGF in SRF of cases with RRD. Our results suggest that several mechanisms contribute to the pathophysiology.

Rhegmatogenous retinal detachment (RRD) is a relatively common cause of ocular morbidity [1,2]. Accumulation of fluid into the potential space between the neurosensory retina and the underlying retinal pigment epithelium (RPE), in the presence of a retinal break or tear, represent the characteristic feature of the disease [3]. The mainstay of treatment is early intervention; restoration of retinal anatomy and blood supply are crucial in this disorder. Functional remodeling of the retina is essential to achieve an acceptable visual recovery [4]

To date, various molecular and cellular mechanisms have been suggested regarding the detachment related retinal ischemia and apoptosis of photoreceptors [5,6]. Several clinical and experimental studies have underlined the role of glutamate in RRD. A significant increase in the levels of glutamate in cases with RRD and the mitigating effect of glutamate receptor antagonists in experimentally induced retinal ischemia have been demonstrated [7,8].

In addition to glutamate, elevation of several inflammatory or growth factor levels have been documented in the vitreous or subretinal fluid (SRF) [1,2,9] of patients with RRD. Among these, vascular endothelial growth factor (VEGF) is recognized with its prominent contribution to the pathophysiology of certain retinal diseases. Essentially, VEGF is a specific mitogen for endothelial cells and an important regulator of angiogenesis. VEGF also exerts pro-inflammatory effects and protects neurons from insults, such as oxidative stress, hypoxia, hypoglycemia, and glutamate-excitotoxicity [10-12]. The relationship between excitotoxicity and induction of VEGF has been interpreted as one of the most interesting pathways that link neurodegeneration with vascular impairment [13]. Considering these interactions, change in amino acid and VEGF concentrations in patients with RRD can be expected.

Intraocular fluid samples, in fact, will provide an accurate basis for verifying these changes. In this study, SRF samples of cases that underwent conventional retinal

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detachment surgery were used and compared with samples of vitreous from non-RRD cases. Besides glutamate, concentrations of a wide spectrum of amino acids and VEGF were analyzed, and the fluctuations of concentrations within the post-detachment period were examined. The results and their relevance to the outcomes of postoperative anatomic and functional success have been studied in detail and are discussed below.

METHODS

Fifty-three patients were included in this university practice-based, prospective, and cross-sectional study within a 19-month period. All patients were of Turkish descent and Caucasian. Cases were otherwise healthy and referred to the ophthalmology clinic of Uludag University. The study group consisted of SRF samples of 46 patients who had undergone scleral buckling surgery with the diagnosis of RRD. Vitreous samples of seven patients who had pars plana vitrectomy (PPV) for full-thickness macular hole (n = 6) or subluxated intraocular lens (n = 1) served as the control specimens. The methodology and the sample group presented herein are similar to a study that we published previously [14]. In that study the correlation between SRF concentrations of S100b protein and the parameters of postoperative success in cases with RRD were investigated. The present study was performed with the approval of the ethics committee of Uludag University School of Medicine, and a written informed consent was obtained from each participant.

In the preoperative examination, all individuals of the study group were asked to meticulously estimate the duration of retinal detachment (DRD). DRD was defined as the time between the onset of symptoms and surgery. Eyes with coexistent ocular disorders, such as vitreous hemorrhage, were excluded from the study. Subjects with bleeding disorders and complicated detachments, e.g., giant retinal tears and proliferative vitreoretinopathy (PVR) grade C1 and higher RRDs, were not included. A history of any prior ocular surgery other than uncomplicated cataract extraction was among the exclusion criteria.

All cases had undergone a follow-up of at least 6 months. Concentrations of VEGF and amino acids were measured in samples of SRF obtained at the time of scleral buckling surgery and compared with vitreous of samples acquired during the PPV of non-RRD cases. The correlations between the overall concentrations of VEGF and amino acids and their change in relation to DRD were further studied. In addition, the interactions between the concentrations and the data noted below of pre- and postoperative examinations were also investigated. Comprehensive ophthalmologic examinations were performed on the day before surgery and on month 6 after surgery.

Preoperative examination parameters comprised an inquiry of DRD, best-corrected visual acuity (BCVA) assessment, slit-lamp biomicroscopy, and fundus examination. Postoperative examination parameters included assessment of BCVA and anterior and posterior segment findings. Anatomic status, presence of postoperative PVR resulting in eyes with recurrent detachment, and additional operations for attachment were recorded at the month 6 of follow-up. BCVA was assessed using the Early Treatment Diabetic Retinopathy Study [15] chart and converted to logarithm of the minimum angle of resolution (logMAR). Depending on DRD intervals, study cases were divided into three subgroups: acute, DRD up to 10 days (n = 13); subacute, DRD longer than 10 days and up to 30 days (n = 23), and chronic, DRD longer than 30 days (n = 10).

All cases underwent SRF drainage before cryotherapy. SRF fluid drainage was performed using a 26-gauge needle attached to a 2-ml syringe without the plunger, as previously described [16]. The needle was inserted perpendicular to the sclera, and the upsurge of fluid was seen in the transparent hub of the needle. After acquiring a minimum of 0.1 ml of SRF, the needle was withdrawn allowing spontaneous drainage of the SRF. Sample volumes ranged from 100 µl to 1 ml. Undiluted vitreous samples of the control group (n = 7) were collected during pars plana vitrectomy before the infusion line was opened. Vitreous was aspirated manually via a 3-cm^3 syringe. Vitreous and SRF samples were stored initially at +4 °C and then moved to −80 °C as soon as possible.

SRF and vitreous fluid controls were analyzed for 15 amino acids, including alanine, aspartate, citrulline, glutamate, glutamine, glycine, isoleucine, leucine, methionine, phenylalanine, serine, taurine, threonine, tyrosine, and valine. Amino acid levels were determined by a high performance liquid chromatography (HPLC) system (HP 1100 series, Hewlett-Packard, Palo Alto, CA), which was coupled to a post-column derivatization unit (Pickering Laboratories, Mountain View, CA). This system was combined with a quaternary pump (HP-G1311A), a fluorometric detector (HP-G1321A), and an autosampler, (HP-G1329A; Hewlett-Packard, Palo Alto, CA). Amino acids were separated on a lithium exchange column (series number 5338; Pickering Laboratories) with Li280 and Li750 eluents (Pickering Laboratories). They were then reacted with o-phthalaldehyde (OPA) in a post-column derivatization unit (both from Pickering Laboratories). The flow rate of the quaternary pump and post-column derivatization unit was 0.3 ml/min. Column

and post-column reaction temperatures were adjusted to 40 °C and 45 °C, respectively. Other chromatographic conditions, such as the gradient program of the Li280, Li750, and lithium regenerant eluents, were similar to those previously published [17]. In accordance, chromatographic elution started with 100% Li 280 for 9 min. Subsequently, percentage of Li 280 decreased as the percentage of Li 750 increased. In the 33rd min of the process, percentage of Li 750 reached to 20%. Before each sample injection, lithium exchange column was regenerated with lithium regenerant eluent for 10 min. OPA reactive compounds were detected using an excitation wavelength of 330 nm and an emission wavelength of 465 nm. The chromatograms were analyzed with HP ChemStation, revision A. 08.03.847 (Agilent Technologies, Waldbronn, Germany). For precipitation of proteins, samples were acidified with equal volumes of Seraprep (Pickering Laboratories) and centrifuged at × 9000 g for 10 min in a Beckman microfuge. The pH of the samples was adjusted to 2.0–2.5 with 2 M of LiOH. A portion of the supernatant (20 µl) was then injected into the HPLC system without further purification. Amino acid levels were calculated by comparing peak heights of the samples with amino acid standards. Amino acid standards were also acidified with Seraprep and processed together with the samples. VEGF levels were measured with human VEGF single bead Luminex kit (Invitrogen Co. Camarillo, CA). Samples were diluted with equal volumes of dilution buffer and assayed according to solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) technique.

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 19.0. (IBM Corp., Armonk, NY) and $p<0.05$ was considered statistically significant. All values were presented in terms of median values with minimums and maximums. Mean values with standard error of means were provided when necessary. The Shapiro-Wilk test was used for the test of normality. Mann-Whitney U tests were used for two-group comparisons, and the Kruskal-Wallis test for three or more group comparisons. Pearson correlation coefficient and Spearman's rank correlation coefficient were used to determine correlations.

RESULTS

Thirty-four male and 19 female patients were enrolled in this study. The study group included SRF samples of 46 patients (29 male, 17 female) that underwent conventional RRD surgery. The mean age of this group was 57±15.9 (range 11–79) years. The control group included vitreous samples of seven patients (five male, two female) who underwent PPV for non-RRD indications. The mean age of the control group was 67.3±3.8 years (range 63–73). Due to technical problems during processing, amino acid levels could not be measured in SRF samples of five cases and VEGF levels could not be obtained in SRF samples of three cases. Fortunately, both amino acid and VEGF levels could be measured in all samples of the control group. In accordance, the amino acid study group consisted of 41 patients (27 male, 14 female), and the VEGF study group consisted of 43 patients (27 male, 16 female). The mean ages of these study groups were 56.9±14.5 (range 11–79) and 56.5±14.2 (range 11–76) years, respectively. There was no significant difference between groups in terms of demographic characteristics, such as age and gender ($p>0.05$).

No significant intraoperative complications, such as subretinal hemorrhage or incarceration, occurred in any participant of the study. Average follow-up was 7.5 + 0.06 months (6 months to 14 months) for the overall study group. Mean DRD was 44.6 days (range 5 days to 18 months). In this study, the overall study group was divided into three subgroups depending on the DRD. The acute subgroup included 13 cases, whereas the subacute and chronic subgroups contained 23 and 10 cases, respectively. In 11 cases (23.9%), postoperative PVR resulted in recurrent detachment. Primary anatomic success rate was 76.1% at the 6th month follow-up. Mean preoperative BCVAs for the overall study group and the control group were 2.05±0.2 and 1.88±1.2 logMAR units, respectively. Mean postoperative BCVA significantly improved to 1.04±0.1 logMAR units in the overall study group ($p<0.001$) and 0.75±0.1 logMAR units in the control group ($p<0.05$).

The levels of amino acids showed a wide variation in this study. Statistical analysis showed that SRF levels were significantly higher for aspartate, citrulline, glutamate, and glycine and lower for alanine, isoleucine, leucine, methionine, phenylalanine, threonine, tyrosine, and valine in the study group ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p = 0.019$, $p = 0.002$, $p = 0.002$, $p = 0.002$, $p = 0.001$, $p = 0.012$, $p = 0.001$, and $p = 0.002$ respectively.) Levels of glutamine, taurine, and serine had no significant change when compared to the control group ($p = 0.139$, $p = 0.358$, and $p = 0.080$, respectively). A comparison of the amino acid levels between the amino acid study group and the control group are presented in Table 1.

Evaluation of amino acids with respect to DRD intervals showed that among the significantly elevated amino acids only glutamate consistently increased throughout the time intervals (Table 2). Median glutamate levels were 59 (7–161) µM in the acute phase, 96 (33–419) µM in the subacute phase, and 139 (60–546) µM in the chronic phase. When the decreasing amino acids were analyzed on the basis

Table 1. Median concentrations with minimum and maximum values of amino acids (mM) and VEGF (pg/ml) of the control group and the study group, and p values.

	Control Group	Study Group	p
Alanine	896 (125-1842)	420 (213-1139)	0.019*
Aspartate	47 (6-70)	84 (0-141)	<0.001*
Citrulline	9 (5-27)	33 (13-102)	<0.001*
Glutamate	20 (10-82)	80 (7-546)	<0.001*
Glutamine	3386 (714-4698)	2618 (1205-4956)	0.139
Glycine	56 (15-196)	154 (66-298)	<0.001*
İsoleucine	220 (39-317)	64 (37-162)	0.002*
Leucine	584 (93-737)	147 (90-407)	0.002*
Methionine	84 (12-133)	22 (10-87)	0.002*
Phenylalanine	356 (66-469)	80 (32-311)	0.001*
Serine	501 (96-733)	379 (0-773)	0.080
Taurine	79 (26-146)	93 (0-172)	0.358
Threonine	385 (77-519)	197 (0-438)	0.012*
Tyrosine	365 (58-399)	99,5 (47-337)	0.001*
Valine	909 (140-1277)	216 (130-710)	0.002*
VEGF	2.5 (1-9.10)	28,5 (1-3130)	<0.001*

* Statistically significant difference between the groups (p<0.05).

Table 2. Median concentrations with minimum and maximum values of amino acids (mM) and VEGF (pg/ml) of different groups of the study, and p values.

	Control	0-10 group	11-30 group	31+ group	p
Alanine	896 (125-1842)	399 (220-677)	496 (213-1139)	378 (248 - 507)	0.030*
Aspartate	47 (6-70)	77 (49-101)	87 (57 - 130)	97 (0 - 141)	0.004*
Citrulline	9 (5-27)	37 (18-92)	33 (13-102)	32 (26-63)	0.002*
Glutamate	20 (10-82)	59 (7-161)	96 (33-419)	139 (60-546)	<0.001*
Glutamine	3386 (714-4698)	2908 (1205-4956)	2660 (1486-3738)	2077 (1295-3602)	0.149
Glycine	56 (15-196)	139 (66-216)	162 (77-298)	192 (93-235)	0.006*
İsoleucine	220 (39-317)	93 (37-162)	67 (53-142)	58 (38-73)	0.005*
Leucine	584 (93-737)	214 (93-407)	160 (113-405)	118 (90-169)	0.003*
Methionine	84 (12-133)	24 (10-71)	24 (16-87)	14 (11-40)	0.003*
Phenylalanine	356 (66-469)	83 (48-259)	91 (32-311)	64 (44-94)	0.002*
Serine	501 (96-733)	322 (179-773)	385 (0-567)	344 (0-464)	0.262
Taurine	79 (26-146)	86 (30-145)	110 (47-172)	80 (0-162)	0.498
Threonine	385 (77-519)	193 (29-438)	208 (0-389)	171 (0-274)	0.030*
Tyrosine	365 (58-399)	109 (52-266)	109 (54-337)	76 (47-121)	0.005*
Valine	909 (140-1277)	310 (131-710)	216 (158.696)	165 (130 - 288)	0.002*
VEGF	2.5 (1-9.10)	25.7 (4.6 - 1775)	25.15 (1-3130)	68.75 (3.6-177.6)	0.004*

* Statistically significant difference between the groups (p<0.05).

of DRD, we observed that the levels of leucine, tyrosine, phenylalanine, and valine steadily declined with time (Table 2). However, levels of alanine showed a prominent decrease only at the chronic stage. Within the time span, levels of glutamine and serine decreased somewhat, whereas levels of taurine increased slightly when compared to the controls; however these changes were statistically insignificant.

Our results showed that median concentrations of VEGF were 28.5 (1–3130) pg/ml in the overall study group and 2.5 (1–9.1) pg/ml in the control group. There was statistically significant difference between the 2 groups (p<0,001). Evaluation of the subgroups demonstrated that median concentrations of VEGF was 25.7 (4.6–1775) pg/ml in the acute stage of RRD (DRD up to 10 days). VEGF levels decreased in the subacute stage (25.1 [1–3130] pg/ml). However, the concentration of VEGF increased to 68.7 (3.6–177.6) pg/ml in the chronic stage. Interestingly, analysis revealed no statistically significant difference in terms of VEGF levels between the three DRD subgroups (p = 0.501).

VEGF levels were correlated with some of the amino acids. Statistically there was a positive and moderate correlation of VEGF levels with glutamate (r = 0.630; p<0.001) and aspartate (r = 0.611; p<0.001). VEGF levels and glycine displayed a weak and positive correlation (r = 0.331; p = 0.034). VEGF and glutamine showed a weak but negative correlation (r = –0.367; p = 0.018).

We could not find any association between the amino acid concentrations and assessments of preoperative or postoperative BCVA. Similarly, no association could be demonstrated between VEGF levels and postoperative PVR or BCVA (p>0.05).

DISCUSSION

Anatomic and functional remodeling of the retina starts soon after retinal detachment [18]. Identifying the ongoing neurochemical processes and monitoring the functional status of the detached retina at any given time is difficult. Continuation of photoreceptor apoptosis in some cases has been underlined as the leading cause of visual loss despite surgical reattachment [19]. In the last decades, intraocular fluids have been used extensively to investigate the contributing mechanisms in retinal diseases, including RRD [20-22]. SRF is unique as it is obtainable only under certain circumstances and serves as a suitable media for investigations due to its close proximity to RPE and photoreceptors.

In general, amino acids constitute the building blocks of proteins and polypeptides in vertebrates. They play key roles in cell signaling and in metabolic pathways that contribute to tissue repair. Amino acids also have significant environmental impacts. Deprivation of some amino acids has been shown to induce endoplasmic reticulum stress and lead to expression of certain growth factors, VEGF in particular. In addition, certain amino acids, such as glutamate, gamma-amino butyric acid (GABA), and glycine, act as the principal neurotransmitters within the retina. Still, several other neuroactive amino acids, including aspartate, homocysteic acid, and taurine, have been identified within the retina, and some amino acid-based neurotransmitters (e.g., dopamine) are used by retinal neurons [23].

The results in this study showed that SRF concentrations of glutamate, aspartate, glycine, citrulline, and VEGF increased significantly in cases with RRD. Glutamate and aspartate are critical amino acids for the central nervous system (CNS). Both amino acids are excitatory neurotransmitters in CNS and are released in a Ca^{+2}-dependent manner. They are removed from the extracellular space via the same glutamate/aspartate transporter; however, they have different affinities for different excitatory amino acid receptor subtypes [24]. Our results showed that SRF levels of glutamate increased consistently as the DRD increased. The highest concentration of SRF glutamate was found in the chronic stage of RRD. Parallel to this, SRF levels of aspartate also increased significantly when compared to the control group. However, levels of aspartate remained fairly constant throughout the time span (Table 2).

In recent years, elaborate studies have been carried out regarding the importance of glutamate in the CNS. It is recognized as the principal excitatory amino acid neurotransmitter within the retina [25]. Homeostasis of glutamate is maintained by an active role of Müller cells. It is mainly synthesized from glutamine by phosphate-activated glutaminase [26]. After its release from neurons, Müller cells uptake the extracellular glutamate. Müller cells subsequently transform glutamate into glutamine by glutamine synthetase [27].

Evidence from previous studies support that excessive levels of glutamate cause excitotoxicity and ultimately lead to DNA damage and subsequent cell death [28]. The toxic effects of glutamate are extensive within the retina. Findings by Sasoh et al. suggest that a high concentration of glutamate is not just limited to Müller cells [29]. In this cat model of retinal detachment, the authors found that an excess amount of glutamate is spread out in a variety of retinal cells, such as bipolar cells, ganglion cells, and ischemic or detached parts of the photoreceptors. Various mechanisms have been proposed in the literature to explain the pathophysiology of excitotoxicity [30-32].

We believe that the high concentrations of glutamate and aspartate in this study represent the excitotoxic state of the retinal neurons and photoreceptors. However, the mechanism of excitotoxicity and the reason why levels of glutamate increased steadily while concentrations of aspartate drew a plateau remains unknown. The significant increase of glutamate was not correlated with an increase of glutamine in the study group. This interesting finding may suggest that the above-mentioned glutamate homeostasis is disturbed at a critical point in order to maintain the levels of glutamine at a certain level.

Another finding in our study showed that SRF levels of glycine increased significantly in RRD. Actually, glycine is found in many parts of the CNS and is plentiful within the retina [18]. Glycine and GABA act as the major inhibitory neurotransmitters within the retina, and glycine is known to exert a significant role in shaping the visual responses of the retina [33-36]. Glycine has a dual neurochemical function; the effect of glycine will vary according to the receptor that it binds. If glycine binds to glycine receptors, it will depress the retinal neurons and cause hyperpolarization by blocking the chloride channels [37,38]. When glutamate binds to the N-Methyl-D-aspartate (NMDA) receptors, it needs glycine to bind at the same time for the receptor to be activated [39]. On this occasion, glycine will act in an excitatory fashion whenever it binds to the NMDA receptor [40]. Our results showed that levels of glycine increased right after the detachment and remained steady throughout the study. Given the above discussed mechanisms, we do not know whether the levels of glycine are elevated as an accompanying process of excitotoxicity or as a preventive response to excitotoxicity. Further studies may clarify this confusion.

In this study, concentrations of SRF citrulline were significantly higher than the concentrations measured in the control group. In nitric oxide (NO)-producing tissues, citrulline is a NO pathway metabolite [28,41]. Elevated concentrations of arginine, citrulline, and nitrite have been demonstrated in vitreous samples of eyes that developed diabetic tractional retinal detachment and RRD [42]. In that study, the authors attributed the increase in the NO pathway metabolites to retinal hypoxia; they suggested that NO may be playing a role in the pathogenesis of RRD. Apparently, elevation of citrulline is a significant finding of our study in terms of showing the diversity of mechanisms contributing to the pathophysiology of rhegmatogenous retinal detachment.

A closer look at the literature reveals that studies on neurologic functions of amino acids are mainly concentrated on glutamate, aspartate, and glycine. However, results presented here demonstrate that other amino acids also undergo a dynamic process of change after RRD (Table 1 and Table 2). We found that SRF concentrations of alanine, isoleucine, leucine, methionine, phenylalanine, threonine, tyrosine, and valine decreased prominently when compared to controls.

In general, tyrosine and phenylalanine function as precursors for the catecholamines (dopamine, norepinephrine, and epinephrine). The dominant catecholamine within the retina is dopamine [43]. Evidence in the literature supports that dopamine acts as a chemical messenger for light adaptation. Dopamine also has trophic roles in retinal function and it is related to circadian rhythmicity, cell survival, and eye growth. It has been previously reported that a reduction in retinal dopamine results in reduced visual contrast sensitivity in patients with Parkinson disease [44]. The consequences of constantly decreasing levels of tyrosine and phenylalanine, as shown in our SRF samples, may be the underlying pathophysiology for impaired contrast sensitivity experienced after RRD. This hypothesis needs to be validated with further studies.

Results of our study demonstrated that the remaining amino acids (glutamine, taurine, and serine) had no prominent change of concentrations when compared to the control group. Animal studies have revealed that taurine is abundant in the retina, vitreous, lens, cornea, iris, and ciliary body of the eye. Ripps et al. suggested that taurine is critical for protecting the distal retina from the toxic levels of glutamate [45]. The relevancy of the latter suggestion with our findings is obscure.

Histologically, VEGF receptors are located on endothelial cells and other non-endothelial retinal cells, including photoreceptors, RPE, and Müller cells. It is known that VEGF is upregulated by certain conditions, such as hypoxia and intraocular inflammation [1,46]. The correlation between some amino acids and VEGF has been examined in certain conditions. Abcouwer et al. demonstrated that RPE cells respond to glutamine starvation by VEGF expression in a similar response to that of hypoxia [47]. Marc et al. noted that Müller cells and RPE cells sequester excitotoxic amino acids to minimize secondary apoptosis [48]. Similarly, it has been suggested that excitotoxic swelling of retinal neurons experienced after ischemia is facilitated by the VEGF-evoked release of glutamate [49]. A clinical study about diabetic retinopathy revealed that an increase in extracellular glutamate is linked with an increase in VEGF expression and blood retinal barrier breakdown [50].

In this study, we investigated whether VEGF expression is increased in RRD and whether levels of SRF amino acids bear any association with VEGF. The results in our study

showed that SRF concentrations of VEGF increased significantly in cases with RRD. Analysis of VEGF concentrations, according to the time frames, revealed that VEGF levels increased soon after RRD; however, it displayed a constant decrease during the acute and subacute phases and then increased within the chronic stage (Table 2). Actually, high levels of VEGF have been demonstrated in neovascularizations secondary to ischemia. Morimozato et al. showed that neovascularization develops whenever VEGF is above the threshold of biologic angiogenesis [51]. Essentially, neovascularization of the iris is seen in long-lasting or inadequately treated retinal detachments. This may be due to the increase in levels of VEGF within the course of the disease, as noted in our study. We think that VEGF is following a parabolic slope as a function of time, and the increasing arm of the slope is in the chronic phase of the disease.

Our results also showed that certain amino acids were related to VEGF. Herein, SRF levels of VEGF were positively correlated with glutamate, aspartate, and glycine. However, there was a negative correlation between VEGF and glutamine. As mentioned above, the aforementioned three amino acids exhibit a close relationship with excitotoxicity. The rising levels of these amino acids and VEGF in our study is consistent with the correlation between excitotoxicity and the induction of VEGF.

We found no evident correlation between BCVA levels and concentrations of VEGF or any of the amino acids. A review of the literature shows only a few publications regarding the SRF levels of amino acids and VEGF. The most important precedent in this regard is the publication of Bertram et al. [22]. Interestingly, our results are identical to those of Bertram et al. except for arginine, which we could not study.

Moreover, herein we report that SRF levels of citrulline increased while methionine and threonine decreased after the onset of RRD. Our results also showed that SRF levels of glutamine, serine, and taurine remained constant when compared to the controls. This paper also draws attention to the fact that there is discordance between the concentrations of glutamate and glutamine. Furthermore, to the best of our knowledge no study has, as yet, focused on the concentration of amino acids in SRF and its relevance with the DRD.

Our data also point out that VEGF concentration in SRF is following a particular time-dependent change pattern and that certain amino acids have a correlation with VEGF levels. Publications regarding the concentrations of VEGF in SRF are few. Ricker et al. found that high levels of VEGF in the subretinal space are related to PVR formation [1]. In our study, contrary to our expectations, we could not find any association between the VEGF levels and postoperative PVR. This difference may be attributed to the selection criteria and the diversity of individuals among the groups.

There are some limitations in our study. We acknowledge that the DRD used in this study is long (mean 44.6 days, range 5 days to 18 months). This may have induced the relatively high rate of PVR that we observed. However, we believe that this wide range also allowed us to examine the change of concentrations as a function of time in a better way. The relatively limited number of cases can be listed as another drawback. Moreover, questions may be raised about the accuracy of a comparison between SRF and vitreous that we used in this study. SRF composition is dynamic and originates from three different sources: vitreous, serum, and the retina. Quintyn et al. underlined that SRF is somewhat closer to vitreous in new onset RRD; however, in older RRD the SRF profile is close to that of serum [52]. To avoid the above-mentioned concerns, concentrations of amino acids and VEGF could have been assessed in samples of serum together with SRF and vitreous. However, we had serious doubts whether these measurements would be related purely to RRD without being affected by other systemic factors. Accordingly, we preferred to use vitreous as the closest medium for comparison.

On the other hand, visual acuity was considered as the sole parameter in evaluating the functional integrity of retina in this study. However, it is well known that BCVA represents the foveal function and does not reflect structural changes of the neurosensory retina outside the foveal region [53]. Therefore, a full assessment of retinal function by integrating optical coherence tomography, microperimetry, multifocal electroretinography, and BCVA might have been more appropriate in this study to determine the value of measurements and their correlation with the functionality of retina.

In conclusion, SRF concentrations of amino acids and VEGF had no significant correlations with the anatomic outcome or BCVA assessments in this study. However, our results have shown that amino acids and VEGF undergo a dynamic process of change after RRD. The diversity presented herein point out that several mechanisms are involved in these processes. Unfortunately, the disclosed aspects of the pathophysiology are still insufficient to predict the individual response to RRD and the subsequent surgery. It appears that timely surgery is requisite for facilitating the recovery of retinal neurons before the devastating effects of excitotoxicity begin. Further studies (e.g., large-scale proteomics-based studies) may reveal the ongoing processes and may contribute to our understanding of the pathogenesis of RRD.

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